A Novel Prognostic Nomogram Accurately Predicts Hepatocellular Carcinoma Recurrence after Liver Transplantation: Analysis of 865 Consecutive Liver Transplant Recipients

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ABSTRACT

Background: While radiological size criteria(Milan/UCSF) have led to improved outcomes following liver transplantation(LT) for hepatocellular carcinoma(HCC), recurrence remains a significant challenge. We analyzed our 30-year experience with LT for HCC to identify predictors of recurrence.

Methods: A novel clinicopathologic risk score and prognostic nomogram predicting posttransplant HCC recurrence was developed from a multivariate competing-risk Cox regression analysis of 865 LT recipients with HCC between 1984 and 2013.

Results: Overall patient and recurrence-free survival were 83%, 68%, 60% and 79%, 63%, and 56% at 1-, 3-, and 5-years. HCC recurred in 117 recipients with a median time-to-recurrence of 15 months, involving the lungs(59%), abdomen/pelvis(38%), liver(35%), bone(28%), pleura/mediastinum(12%), and brain(5%). Multivariate predictors of recurrence included tumor grade/differentiation(G4/poor diff HR 8.86; G2-3/mod-poor diff HR 2.56), macrovascular(HR 7.82) and microvascular(HR 2.42) invasion, non-downstaged tumors outside Milan criteria(HR 3.02), non-incidental tumors with radiographic maximum diameter > 5cm(HR 2.71) and < 5cm(HR 1.55), and pretransplant neutrophil-to-lymphocyte ratio(HR 1.77 per log unit), maximum alphafetoprotein(HR 1.21 per log unit), and total cholesterol(HR 1.14 per SD). A pretransplant model incorporating only known radiographic and laboratory parameters had improved accuracy in predicting HCC recurrence(c-statistic 0.79) compared to both Milan(c-statistic 0.64) and UCSF(c-statistic 0.64) criteria alone. A novel clinicopathologic prognostic nomogram included explant pathology and had an excellent ability to predict posttransplant recurrence (c-statistic 0.85).

Conclusions: In the largest single-institution experience with LT for HCC, excellent long-term survival was achieved. Incorporation of routine pretransplant biomarkers to existing radiographic size criteria significantly improves the ability to predict posttransplant recurrence, and should be

considered in recipient selection. A novel clinicopathologic prognostic nomogram accurately predicts HCC recurrence after LT and may guide frequency of posttransplant surveillance and adjuvant therapy.

Abbreviations and Acronyms

HCC- Hepatocellular carcinoma

LT- Liver transplantation

MC- Milan criteria

UCSF- University of California, San Francisco

MELD- Model for End-Stage Liver Disease

AFP- Alphafetoprotein

NLR- Neutrophil to lymphocyte ratio

BMI- Body mass index

IQR- Interquartile range

AJCC- American Joint Committee on Cancer

NASH- Nonalcoholic steatohepatitis

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third most common cause of cancer-related death(1). In the United States, the incidence of HCC has nearly doubled over the last 2 decades(2-4). While the majority of patients present with locally advanced or metastatic disease, early stage patients may be candidates for potentially curative surgical therapy, including resection and liver transplantation (LT)(5). LT provides a complete oncologic resection while simultaneously replacing the diseased liver, a predisposing factor in more than 90 percent of patients with HCC.

Despite the logic of this approach, the early results of LT for HCC were plagued with prohibitive tumor recurrence and mortality(6-9), largely due to poor patient selection. The so-called Milan criteria (MC), introduced in 1996, limited LT to patients with a single tumor of 5 cm diameter or less or up to three tumors, none larger than 3 cm(10). Application of MC resulted in excellent post-transplant recurrence-free survival and solidified LT as the gold-standard therapy for patients with underlying liver dysfunction and tumors meeting these specified size criteria. In an attempt to extend the life-saving benefit of LT, numerous subsequent criteria have been proposed, allowing for transplantation of a larger size and number of tumors and reporting survival comparable to the MC(11-13).

With the introduction in 2002 of the Model for End-Stage Liver Disease (MELD) liver allocation system(14), which allows for prioritization of HCC recipients with tumors meeting radiographic size criteria, the frequency of LT for HCC has nearly doubled(15). Currently, HCC is the indication for LT in nearly a quarter of adult liver recipients in the US. Despite nationwide adoption of the Milan/UCSF radiographic size criteria, HCC recurrence after transplantation remains a significant cause of graft loss and mortality, affecting up to 8-18% of recipients(16, 17). This is explained in part by the recognition that radiographic size is only a rough surrogate for the key pathologic characteristics that define tumor biology, including tumor grade/differentiation and vascular invasion(16, 17).

The current study reports a large, single-center experience of LT for HCC spanning three decades. We sought to identify important multivariate predictors of HCC recurrence and to develop a novel clinicopathologic prognostic nomogram incorporating radiographic, laboratory, and pathologic characteristics that can be used to accurately predict the risk of post-transplant HCC recurrence and guide adjuvant therapy.

METHODS

We performed a retrospective review of a prospectively maintained transplant database and identified all adult patients (age 18 years and older) who underwent LT for HCC or were incidentally discovered to have HCC on explant pathology at the University of California, Los Angeles from 1984 to 2013. Multiple recipient (age, gender, primary end-stage liver disease diagnosis, diabetes, hypertension, hyperlipidemia, coronary artery disease), donor and operative (donor age, gender, heartbeating cadaveric, non-heart beating cadaveric, split graft, cold ischemia time, warm ischemia time), laboratory (alphafetoprotein-AFP, neutrophil-to-lymphocyte ratio-NLR, MELD score, total cholesterol) pretransplant radiographic (number of lesions, maximal tumor diameter, cumulative tumor diameter), and pathologic (number of lesions, maximal tumor diameter, cumulative tumor diameter, T stage, grade, differentiation, and vascular invasion) variables were analyzed to determine predictors of HCC recurrence after OLT. This study was approved by the UCLA Institutional Review Board.

Pretransplant disease extent was determined based on computed tomography (CT) or magnetic resonance (MR) images. Patients with HCC were classified as having tumors within MC, beyond MC but within UCSF criteria, or exceeding UCSF criteria. Patients without a pretransplant radiographic diagnosis of HCC who had an incidental HCC discovered on explant were categorized as within radiographic MC. Pretransplant adjuvant treatments including chemotherapy, thermal ablation (radiofrequency or microwave ablation), transarterial

embolizations (bland transarterial embolization, chemoembolization, radioembolization), and liver resections were used in select patients. Patients beyond MC were further characterized based on the ability of adjuvant treatments to downstage them into MC prior to LT.

All liver explants were examined by an experienced hepatopathologist and categorized based on tumor number, size, distribution, histologic grade and differentiation(18), presence of micro- and macro-vascular invasion, perineural invasion, and lymph node involvement. The American Joint Committee on Cancer (AJCC) T stage was recorded(19).

Statistical Analysis

For descriptive statistics, the number of patients with a given characteristic is reported. The percentages were calculated excluding patients with missing data for any given variable. Most variables had no or few (<5%) missing data. The following variables had missing values > 5%: pathologic tumor grade (14%) and differentiation (14%) due to complete tumor necrosis on explant, BMI (8%), NLR (7%), and MELD (7%). For the multivariate analysis, missing values were singly imputed by the Markov chain Monte Carlo method, allowing for arbitrary missing data patterns.

Primary outcome was the time to HCC recurrence after OLT, with recurrence-free and overall survival as secondary outcomes. Survival curves were computed Kaplan-Meier methods and compared using log-rank tests. Cumulative probabilities of recurrence were calculated while taking into account the competing risk of non-HCC related mortality. Univariate analysis of individual predictors of HCC recurrence was performed using the Fine and Gray competing risks Cox regression model. Factors identified as significant ($P < 0.25$) on univariate analysis were entered into a multivariate competing risk Cox regression model to identify significant independent predictors of HCC recurrence.

The final multivariate model was performed using the backwards stepwise procedure for variable selection with a liberal $P < 0.15$ as the retention criteria. The NLR and AFP values had a skewed distribution on the original scale, and were transformed to the logarithmic scale where they displayed a normal distribution. Hence, the logarithmic transformed variables were used for the multivariate analysis. Linearity was confirmed using restricted cubic splines. Results are summarized using the unadjusted (univariate) and adjusted (multivariate) hazard ratios (HR), 95% confidence intervals, and P-values. The hazard ratios for continuous variables were reported per standard deviation for the forrest plot, and per log unit for the risk calculator.

Model accuracy was summarized using the concordance "C" statistic(20), and model validation was performed using 10-fold cross validation with the validated C statistic reported. An HCC recurrence risk score (R) was developed from the weighted sum of each independent predictor, with the weights equal to the regression coefficient (log hazard ratio) from the multivariate model. The risk score could be used to calculate the predicted risk of recurrence for a given patient at time (t) using the following equation: $\text{Risk}(t) = (1- (1- \text{Risk}_0(t))^{\exp(R-R0)}$, where Risk_0 is the estimated risk of recurrence for an average patient at time t obtained from the cumulative incidence curve, and R0 is the average risk score, 2.39. Finally, a clinicopathologic prognostic nomogram was generated based on the competing risk model using the "rms" library in R (R project for statistical computing, http://www.r-project.org/).

RESULTS

During the study period, 865 adult patients with HCC underwent LT. Median age was 60, and 73% were male. Median follow up time was 29.7 months (IQR, 9.1-73.0).

Recipient characteristics and pretransplant tumor treatment

Baseline demographic, laboratory and radiologic characteristics of recipient and tumors are shown in Table 1. Hepatitis C was the most common underlying diagnosis (58%), followed by hepatitis B (16%), alcoholic liver disease (9%), and nonalcoholic steatohepatitis (4%). The

median laboratory MELD score was 14 (IQR 10-22), pretransplant maximum AFP was 22 (IQR 6-121), immediate pretransplant AFP was 11 (IQR 5-50), NLR was 3.1 (IQR 1.9-5.4), and total cholesterol was 144 (IQR 116-174). On pretransplant radiographic imaging, 476 patients (56%) had 1 lesion, while 120 (14%), 49 (6%), and 20 (2%) had 2, 3, and 4 or more lesions, respectively. HCC was not detected on imaging and was an incidental finding on explant pathology in 187 patients (22%). By radiologic size criteria, 84% and 92% of tumors were within Milan and UCSF criteria, respectively, with only 8% outside of UCSF criteria.

Pretransplant HCC treatment was performed in 516 of 865 LT recipients (Table 2). Of these 516 patients, 282, 148, 50, and 32 underwent 1, 2, 3, and 4 or more treatments, including transarterial embolization (36%), percutaneous thermal ablation (29%), percutaneous ethanol ablation (3%), liver resection (3%), and chemotherapy (2%). Of 717 patients within MC, 405 (57%) received bridging therapy prior to transplantation. Of 140 patients outside MC, 71 (51%) were downstaged to MC with pretransplant treatment, while 69 (49%) were not. Of the 71 patients outside MC who were downstaged to MC, 60 (85%) were within UCSF criteria while 11 (15%) were outside UCSF. Of the 69 patients not downstaged to MC, only 12 (17%) were within UCSF criteria, 57 (83%) were outside UCSF criteria, and 35 (51%) did not receive HCC treatment.

Pathologic tumor characteristics

Explant pathology is shown in Table 3. Of 865 patients, 447 had 1 tumor (52%), while 168 (20%), 102 (12%), 52 (6%), and 85 (10%) had 2, 3, 4, and 5 or more lesions. Vascular invasion was absent in 646 patients (75%), with 163 (19%) and 55 (6%) demonstrating microvascular and macrovascular invasion, respectively. The majority of patients had nuclear grade 1 (21%) or 2 (55%) tumors, corresponding to the well (26%) and moderately (53%) differentiated tumors. Nuclear grade 3 (21%) and 4 (3%) tumors accounted for all of the poorly differentiated (21%) HCCs. Stratified by the AJCC T stage, 385(44%), 344(40%), 76(9%), 50(6%), and 9(1%) had T1, T2, T3a, T3b, and T4 tumors, respectively.

Outcomes

Following LT, 117 of 865 recipients had recurrence of HCC, with a median time-to-recurrence of 15 months. Sites of recurrence included the lungs (59%), abdomen/pelvis (38%), liver (35%), bone (28%), pleura/mediastinum (12%), and brain (5%). Overall and recurrence-free survival for the entire group at 1, 3, and 5 years after transplantation was 83%, 68%, 60% and 79%, 63%, and 56%, with a cumulative incidence of non-HCC death of 15%, 24%, 29% and HCC recurrence of 6%, 13%, 15% (Figure 1).

Recurrence-free survival by radiologic size criteria and response to downstaging is shown in Figure 2A. Patients within MC or downstaged to MC had similar survival at 1, 3, and 5 years of 81%, 67%, 60% and 85%, 62%, 55%, significantly superior to the 63%, 33%, and 27% survival observed in recipients beyond MC who were not successfully downstaged prior to transplantation ($p < 0.001$). For patients within MC, recipients bridged to LT with pretransplant HCC treatment had significantly superior 1, 3, and 5 year survival compared to HCC patients not bridged to LT (85%, 71%, 63% vs 72%, 61%, 53%, p=0.001; Figure 2B).

Recurrence-free survival by pathologic vascular invasion is shown in Figure 3. Recurrence-free survival for patients without vascular invasion was 84%, 71%, and 64% at 1, 3, and 5 years, significantly superior to recipients with microvascular invasion (73%, 50%, and 44%) and macrovascular invasion (49%, 23%, and 13%, $p<0.001$). The AJCC pathologic T stage was also a good discriminator of recurrence-free survival (Figure 4). When stratified by T stage, recipients with T1, T2, T3a, and T3b/T4b tumors had survival outcomes of 83%, 83%, 76%, and 47% at 1 year; 72%, 66%, 48%, and 22% at 3 years; and 64%, 60%, 41%, and 12% at 5-years ($p<0.001$).

HCC recurrence

Univariate predictors of HCC recurrence are shown in Tables 4 and 5. Tumors were significantly less likely to recur in recipients older than 55 years (HR 0.62, p=0.009), with hepatitis C (HR 0.56, p=0.017) and MELD > 10 (HR 0.64, p=0.031), and significantly more likely to recur in patients

with greater pretransplant total cholesterol (HR 1.003 per mg/dL, p=0.033), AFP (HR 1.91 per log unit, p<0.001), maximum AFP (HR 1.82 per log unit, p<0.001) and NLR (HR 2.14 per log unit, p=0.002). No other recipient, laboratory, or donor and operative variables conferred an increased risk of HCC recurrence.

Radiographic predictors of recurrence included number of lesions (1- HR 2.67, p=0.004; 2- HR 4.67, p<0.001; 3 or more- HR 7.73, p<0.001), maximum (HR 13.6 per log unit, p<0.001) and cumulative tumor diameter (HR 15.0 per log unit, p<0.001), non-downstaged tumors outside MC (HR 10.2, p<0.001), bilobar lesions (HR 2.88, p<0.001) and the need for 3 or more tumor treatments (HR 2.66, p<0.001). Pathologic predictors of recurrence included number of lesions (2- HR 2.04, p=0.008; 3 or more – HR 3.63, p<0.001), maximum (HR 34.4 per log unit, p<0.001) and cumulative (HR 62.6 per log unit, p<0.001) tumor diameter, microvascular (HR 2.98, p<0.001) and macrovascular (HR 17.3, p<0.001) invasion, nuclear grade 3/poor differentiation (HR 5.28, p<0.001) and nuclear grade 4/poor differentiation (HR 13.2, p<0.001), multifocality (HR 3.10, p<0.001), and AJCC T stage (T2- HR 2.88, p=0.001; T3a- HR 9.59, p<0.001; T3b- HR 31.7, p<0.001; T4- HR 91.8, p<0.001).

Multivariate Analysis and Risk Models

Multivariate predictors of recurrence (Figure 5) include nuclear grade 4/poor differentiation (HR 8.86, 95% CI 3.37-23.3, p<0.001), macrovascular invasion (HR 7.82, 95% CI 4.69-13.1, p<0.001), non-downstaged tumors beyond MC (HR 3.02, 95% CI 1.66-5.49, p<0.002), non-incidental tumors with maximum radiologic diameter > 5cm (HR 2.71, 95% CI 1,31-5.62, p=0.007), nuclear grade 2-3/moderate to poor differentiation (HR 2.56, 95% CI 1.26-5.17, p<0.001), microvascular invasion (HR 2.42, 95% CI 1.49-3.94, p<0.001), non-incidental tumors with maximum radiologic diameter < 5cm (HR 1.55, 95% CI 0.87-2.75, p=0.136), NLR (HR 1.77 per log unit, 95% CI 1.08-2.89, p=0.023), pretransplant maximum AFP (HR 1.21 per log unit, 95% CI 1.01-1.45, p=0.037), and total cholesterol (HR 1.003 per mg/dL increase, 95% CI 0.999-1.006, p=0.161).

Two risk prediction models based on these multivariate models were developed. A pretransplant model included only ascertainable pretransplant variables and is shown in Table 6. Significant predictors of HCC recurrence included tumors beyond MC which were not downstaged to MC (HR 4.96, 95% CI 2.92-8.44, p<0.001), non-incidental tumors with maximum radiologic diameter > 5cm (HR 1.85, 95% CI 0.9-3.79, p=0.093) and < 5cm (HR 1.60, 95% CI 0.91-2.80, p=0.102), NLR (HR 1.89 per log unit increase, 95% CI 1.15-3.10, p=0.012), pretransplant maximum AFP (HR 1.48 per log unit increase, 95% CI 1.23-1.78, p<0.001), and total cholesterol (HR 1.003 per mg/dL increase, 95% CI 0.999-1.007, p=0.108). This model had a nominal c-statistic of 0.79 (95% CI 0.75-0.83) and a 10-fold cross-validated c-statistic of 0.78 (95% CI 0.70-0.85) in predicting HCC recurrence, significantly superior to the ability of both the Milan (c-statistic 0.64, 95% CI 0.59-0.68) and UCSF (c-statistic 0.64, 95% CI 0.59-0.68) criteria to predict HCC recurrence (p<0.001 for both pairwise comparisons).

A second model utilized all clinicopathologic multivariate predictors (Figure 5) to develop a risk score to predict HCC recurrence. The risk score for an individual patient is the weighted sum of the individual predictors with weights equal to the regression coefficients (log hazard ratio) in the final model and is calculated as follows: R = 2.18 * (1 if grade 4/poorly differentiated) + 2.06 * (1 if macrovascular invasion) + 1.10 * (1 if non downstaged and beyond Milan) + 1.00 * (1 if non-incidental and radiologic maximum diameter > 5cm) + 0.94 * (1 if grade 2-3/moderate-poor differentiation) + 0.88 * (1 if microvascular invasion) + 0.44 * (1 if non-incidental and radiologic maximum diameter < 5cm) + 0.57 * (log10 NLR) + 0.19 * (log10 pretransplant maximum AFP) + 0.003 * (total cholesterol in mg/dL). The risk score accurately predicted the risk of HCC recurrence (Figure 6) with a c-statistic of 0.85 (95% CI 0.82-0.89) and a 10-fold cross-validated c-statistic of 0.84 (95% CI 0.76-0.92), significantly superior to the ability of the AJCC T staging system (c-statistic 0.80, 95% CI 0.75-0.83, p=0.006). A novel clinicopathologic prognostic nomogram was developed to predict the 1-, 3-, and 5-year risk of recurrence for any individual patient with HCC (Figure 7).

DISCUSSION

Of the first seven orthotopic liver transplants, six were performed for primary or secondary liver malignancies, including three for HCC, the most common primary liver cancer(21). More than half a century later, the logic of replacing a liver burdened with tumor is largely unchanged: to provide the best oncologic resection and simultaneously correct the underlying liver dysfunction. While transplantation has improved with significant technical, perioperative and immunological advances over its first 30 years, oncologic outcomes after LT for HCC remained dismal prior to the adoption of the Milan criteria in 1996(10), which limited LT to recipients whose tumors were confined by size criteria.

Despite improvements in recurrence-free survival with utilization of the MC(16, 22), post-transplant recurrence remains a significant clinical problem(17). These accepted radiologic criteria are only rough surrogates for the underlying tumor biology, and fail to incorporate other known laboratory and pathologic characteristics that predict tumor behavior. With this largest single-center report of LT for HCC, we identify important multivariate predictors of post-transplant HCC recurrence and propose a pretransplant model utilizing only known preoperative factors that augments the ability of the existing radiologic criteria to select HCC patients at low risk of recurrence. Additionally, we develop a novel comprehensive clinicopathologic prognostic nomogram that accurately predicts an individual patient's risk of recurrence and may be used to guide the frequency of post-transplant surveillance and use of adjuvant therapy.

Many prior studies have demonstrated improved oncologic and survival outcomes when LT is limited to patients whose tumors are confined by radiological size criteria(10-13, 16, 23). While pretransplant locoregional treatment of these tumors, so called bridging therapy, has been widely accepted to prevent tumor progression and wait-list dropout(24-28), data on posttransplant survival benefits have been conflicting. Despite an apparent reduction in post-transplant HCC recurrence in recipients within MC receiving pretransplant bridging therapy(27-29), significant

improvements in disease-free survival have not been consistently demonstrated(30-36), potentially because the studies have been underpowered. In the current study, recipients within MC who received bridging therapy demonstrated superior 1, 3, and 5-year recurrence-free (Figure 2B) and overall survival (86%, 74%, 68% vs 75%, 63%, 57%, p=0.003) compared to patients not receiving bridging therapy. However, the use of bridging therapy in patients within MC was surprisingly not a predictor of recurrence in either univariate or multivariate analysis. These observed differences in survival may be due to an increase in non-HCC related mortality in recipients not receiving bridging therapy, who had significantly greater pretransplant acuity (MELD score 22 vs 14, $p<0.001$) that likely explained the decision to not pursue treatment.

Our data more convincingly support the importance of successful downstaging therapy. We show that recipients originally beyond MC and successfully downstaged to MC had equivalent recurrence-free survival at 1, 3, and 5-years compared to patients originally within MC and significantly superior survival compared to patients beyond MC who were not downstaged (Figure 2A). These observed survival differences were attributable to cancer recurrence, as the inability to downstage recipients beyond MC strongly predicted HCC recurrence in both univariate and multivariate analysis. Our findings are consistent with prior studies(37-44). While it is difficult to definitively attribute the improved post-transplant outcomes to the tumor necrosis achieved by downstaging, we do feel that the ability to downstage tumors is a surrogate for a more favorable underlying tumor biology. This contention is supported by the fact that microvascular invasion was significantly greater in recipients beyond MC who could not be downstaged, compared to recipients downstaged to MC (49% vs 22%, p=0.012). Morevoer, 11 of the 71 patients that were successfully downstaged to MC were originally outside of UCSF criteria. Taken collectively, these findings underscore the importance of modifying the current prioritization schemes so that potentially life-saving transplants are not denied on the basis of size criteria alone.

Over the last decade, there has been accumulating evidence that pretransplant serum biomarkers can predict cancer recurrence in patients undergoing LT for HCC. The neutrophil-lymphocyte ratio (NLR), an indicator of systemic inflammatory status, has been recognized as a prognostic indicator in various malignancies(45-49). Halazun et al reported that HCC recipients with a pretransplant NLR > 5 had a significantly increased incidence of post-transplant HCC recurrence (62% vs 14%, $p<0.001$) and inferior recurrence-free and overall(50). Serum alphafetoprotein has also been widely recognized as a marker for poor prognosis after LT(23, 51-53), and models incorporating its use in addition to radiologic size criteria for the selection of HCC recipients have been proposed. Hameed et al showed that using an AFP level > 1000 ng/mL would exclude 4.7% of patients from LT but achieve a 20% reduction in HCC recurrence(54). The Liver Transplantation French Study Group developed and validated a model combining AFP with tumor size and number that was significantly superior to MC alone in predicting posttransplant HCC recurrence and survival(52). In our study, both pretransplant maximum AFP and NLR were significant predictors of HCC recurrence, with the recurrence risk increasing linearly with log increases for both markers.

With this increasing recognition that size and number criteria alone do not best predict the risk of HCC recurrence, we developed a pretransplant model utilizing only known patient and tumor characteristics prior to LT. In addition to existing radiologic criteria, pretransplant NLR, AFP, and total cholesterol were independent predictors of post-transplant recurrence (Table 6). Incorporation of these easily available laboratory parameters significantly improved our model's ability to predict post-transplant recurrence and death (c-statistic 0.79 and 0.61, respectively), compared to both the Milan (c-statistic 0.64 and 0.53) and UCSF (c-statistic 0.64 and 0.52) criteria alone. While our pretransplant model needs to be validated prospectively, it provides further evidence along with prior reports that a rigid one-size (and number) policy does not fit all.

Although pretransplant models incorporating serum biomarkers improve upon radiologic size criteria in prognosticating HCC outcomes after LT, the importance of pathologic characteristics

cannot be overstated. Numerous prior studies have demonstrated that HCC tumor grade/differentiation and the presence of vascular invasion are among the most important factors predicting tumor behavior(16, 17). The challenge has been to accurately characterize these pathologic features prior to LT. In a study by Pawlik et al(55), preoperative needle core biopsy (NCB) significantly underestimated the presence of poor differentiation on the final resection or explant specimen (15.1% poor differentiation on NCB vs 27.9% on surgical specimen, $p<0.05$). Furthermore, while macrovascular invasion may be detected on preoperative imaging to exclude LT candidacy, reliably determining microvascular invasion has not been possible, as pathologic grade based on NCB does not appear to correlate with microvascular invasion(55). Based on these inconsistencies between the pretransplant biopsy and final pathology, the routine use of pretransplant biopsy to inform patient selection has not been widely adopted.

The development of prognostic nomograms in the management of malignancies are useful tools to calculate individualized risks of cancer recurrence, and can be used to guide the frequency of post-surgical surveillance and adjuvant therapy. In 2007, Cho and colleagues created a novel prognostic nomogram to predict recurrence-free survival (c-statistic 0.67) and overall survival (c-statistic 0.74) after resection of hepatocellular carcinoma, which demonstrated a markedly superior concordance statistic when compared to numerous other staging systems(56). Shim et al also proposed a prognostic nomogram to predict 2-yr recurrence (c-statistic 0.66) and 5-year disease specific survival (c-statistic 0.67) after resection of hepatocellular carcinoma, identifying gender, serum albumin, platelet count, microvascular invasion, and calculated tumor volume as independent predictors(57). To our knowledge, we report here the first prognostic nomogram for predicting recurrence after liver transplantation for HCC. Our comprehensive prognostic nomogram utilized all clinicopathologic variables including explant data to accurately predict post-transplant recurrence (c=0.85), superior to the ability of the existing AJCC staging system (c=0.80) as well as prior nomograms predicting recurrence after resection. In addition to the radiological and laboratory parameters in our pretransplant model, we identified pathologic tumor grade and vascular invasion as significant independent predictors.

The Milan criteria represented a major step in improving the outcomes of HCC recipients undergoing LT. However, there is now a growing consensus and body of evidence that these criteria are too conservative, and that incorporation of other radiologic tumor and pretransplant laboratory factors may improve the ability to select for patients with favorable tumor biology, regardless of size, who stand to benefit from liver transplantation. With the largest reported single-center experience of LT for HCC, we propose a pretransplant model that incorporates serum biomarkers (AFP, NLR, and cholesterol) in addition to radiologic criteria and significantly improves upon the ability to predict post-transplant recurrence compared to the established size criteria alone. Finally, we have developed a novel and practical clinicopathologic nomogram that accurately predicts post-transplant recurrence, and can be used to guide the frequency of post-transplant surveillance as well as adjuvant therapy in patients at high risk of post-transplant recurrence.

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Table 1. Baseline recipient characteristics	
Demographic characteristics	
Age, median (IQR)	59.5 (53.8-64.6)
Gender	
Male, n (%)	634 (73.3%)
Female, n (%)	231 (26.7%)
BMI, kg/m^2, median (IQR)	26.6 (24.0-30.5)
Diagnosis, n (%)	
Hepatitis C	505 (58)
Hepatitis B	134 (16)
Alcohol	80 (9)
NASH	33 (4)
Cryptogenic	30 (3)
Other	83 (10)
Laboratory parameters	
Laboratory MELD score, median (IQR)	14 (10-22)
Maximum AFP, ng/mL, median (IQR)	22 (6-121)
Immediate pre-LT AFP, ng/mL, median (IQR)	11 (5-50)
NLR, median (IQR)	3.1 (1.9-5.4)
Total cholesterol, mg/dL, median (IQR)	144 (116-174)
Radiologic tumor characteristics	
Number of lesions, n (%)	
0 (incidental on explant)	187 (22)
1	476 (56)
2	120 (14)
3	49 (6)
4+	20 (2)
Within Milan criteria, n (%)	717 (84)
Within UCSF criteria, n (%)	789 (92)
Outside UCSF criteria, n (%)	68 (8)

IQR, interquartile range; NASH, nonalcoholic steatohepatitis; AFP, alphafetoprotein; NLR, neutrophil-lymphocyte ratio; MELD, Model for end-stage liver disease

Table 2. Pretransplant tumor treatment	
Any treatment, n (%)	516 (60)
Number of pre-LT treatments, n (%)	
1	282 (33)
2	148 (17)
3	50 (6)
4+	32 (4)
Type of pre-LT treatment, n (%)	
Transarterial embolization	313 (36)
Percutaneous thermal ablation	253 (29)
Percutaneous ethanol ablation	26 (3)
Liver resection	26 (3)
Chemotherapy	17 (2)
Within Milan criteria, n	717
Bridged to LT, n (%)	405 (57)
Outside Milan criteria, n	140
Downstaged to Milan, n (%)	71 (51)
Not downstaged to Milan, n (%)	69 (49)

Table 3. Explant pathology characteristics	
Number of lesions, n (%)	
1	447 (52)
2	168 (20)
3	102 (12)
4	52 (6)
5+	85 (10)
Vascular invasion, n (%)	
None	646 (75)
Microvascular	163 (19)
Macrovascular	55 (6)
Nuclear grade, n (%)	
1	160 (21)
2	405 (55)
3	155 (21)
4	20 (3)
Differentiation, n (%)	
Well	195 (26)
Moderate	392 (53)
Poor	153 (21)
AJCC T stage, n (%)	
T1	385 (44)
T2	344 (40)
T3a	76 (9)
T3b	50 (6)
T4	9 (1)

AJCC, American Joint Committee on Cancer

Table 4. Univariate analysis of recipient, donor, and operative factors on HCC recurrence

	HR	95% CI	p-Value
Recipient characteristics			
Age > 55	0.62	0.43-0.89	0.009
Male	1.32	0.85-2.03	0.215
Diabetes	0.81	0.52-1.25	0.336
Hypertension	0.86	0.55-1.34	0.504
CAD	0.44	0.11-1.83	0.261
Dialysis	0.50	0.07-3.81	0.504
Past smoker	1.21	0.81-1.80	0.344
Current smoker	0.86	0.37-1.98	0.716
Diagnosis			
Hepatitis B	1.00	ref	ref
Hepatitis C	0.56	0.34-0.90	0.017
Alcohol	0.73	0.36-1.48	0.383
NASH	0.34	0.08-1.41	0.137
Cryptogenic	0.89	0.34-2.35	0.811
Laboratory parameters			
MELD > 10	0.64	0.43-0.96	0.031
Total cholesterol, per mg/dL	1.003	1.000-1.006	0.033
Log pre-LT AFP, per log unit	1.91	1.62-2.24	<0.001
Log pre-LT AFP max, per log unit	1.82	1.54-2.15	<0.001
Log NLR, per log unit	2.14	1.31-3.50	0.002
Donor and operative variables			
Non standard graft*	1.47	0.87-2.48	0.154
Donor age, per year	1.00	0.99-1.01	0.492
Donor male	1.30	0.87-1.95	0.204
Cold ischemia time > 10 h	1.13	0.68-1.90	0.636
Warm ischemia time > 45 min	1.20	0.82-1.90	0.347

CAD, coronary artery disease; NASH, nonalcoholic steatohepatitis, AFP, alphafetoprotein; NLR, neutrophil-to-lymphocyte ratio; *includes nonheartbeating cadaveric and split grafts

Table 5. Univariate analysis of radiographic and pathologic factors on HCC recurrence

	HR	95% CI	p-Value
Radiographic characteristics			
Number of lesions			
0 (incidental on pathology)	1.00	ref	ref
1	2.67	1.36-5.24	0.004
2	4.67	2.22-9.79	<0.001
3+	7.73	3.78-15.8	<0.001
Maximum diameter, per log unit	13.6	6.14-30.0	<0.001
Cumulative diameter, per log unit	15.0	6.53-34.7	<0.001
Radiographic size criteria			
Within Milan, not bridged to LT	1.00	ref	ref
Within Milan, bridged to LT	1.28	0.78-2.11	0.322
Outside Milan, downstaged	1.29	0.57-2.92	0.545
Outside Milan, not downstaged	10.2	6.29-16.5	<0.001
Bilobar	2.88	1.88-4.42	<0.001
Pre-LT HCC treatment	1.18	0.81-1.71	0.388
Pre-LT number of treatments			
0	1.00	ref	ref
1	1.06	0.68-1.67	0.798
2	1.20	0.70-2.05	0.499
3+	2.66	1.58-4.47	<0.001
Pathologic characteristics			
Number of lesions			
1	1.00	ref	ref
2	2.04	1.21-3.44	0.008
3+	3.63	2.38-5.55	<0.001
Maximum diameter, per log unit	34.4	14.3-82.6	<0.001
Cumulative diameter, per log unit	62.6	26.4-148	<0.001
Vascular invasion			
None	1.00	ref	ref
Microvascular	2.98	1.88-4.71	<0.001
Macrovascular	17.3	11.4-26.3	<0.001
Tumor nuclear grade/differentiation			
1/well	1.00	ref	ref
2/well	0.55	0.07-4.39	0.569
2/moderate	3.23	1.61-6.47	0.001
3/moderate	3.05	0.80-11.6	0.103
3/poor	5.28	2.53-11.0	<0.001
4/poor	13.2	4.84-36.2	<0.001
Multifocal	3.10	2.06-4.65	<0.001
AJCC T stage			
T1	1.00	ref	ref
T2	2.88	1.55-5.35	0.001
T3a	9.59	4.93-18.7	<0.001
T3b	31.7	17.1-58.7	<0.001
T4	91.8	41.2-204	<0.001

LT, liver transplantation; AJCC, American Joint Committee on Cancer

Table 6. Pretransplant multivariate model to predict posttransplant HCC recurrence			
	HR	**95% CI**	**p-Value**
Radiographic characteristics			
Within Milan or Outside Milan and downstaged to Milan	1.00	ref	ref
Outside Milan, not downstaged	4.96	2.92-8.94	<0.001
No radiographic lesion (incidental)	1.00	ref	ref
Radiologic maximum tumor diameter < 5cm	1.60	0.91-2.80	0.102
Radiologic maximum tumor diameter > 5cm	1.85	0.90-3.79	0.093
Laboratory characteristics			
NLR, per log unit	1.89	1.15-3.10	0.002
Pretransplant maximum AFP, per log unit	1.48	1.23-1.78	<0.001
Total cholesterol, per mg/dL	1.003	0.999-1.007	0.108
NLR, neutrophil-lymphocyte ratio; AFP, alphafetoprotein; Model C-statistic = 0.79			

Figure Legends

Figure 1. Cumulative incidence curves demonstrating overall survival, recurrence-free survival, death not related to hepatocellular carcinoma, and posttransplant HCC recurrence at 1-, 3-, and 5-years after liver transplantation.

Figure 2. Kaplan-Meier recurrence-free survival curves with 1-, 3-, and 5-year estimates comparing (A) recipients within Milan criteria (MC), Outside MC and downstaged to MC, and Outside MC not downstaged to MC and (B) recipients within MC with and without prior bridging pretransplant HCC treatment.

Figure 3. Kaplan-Meier recurrence-free survival curves with 1-, 3-, and 5-year estimates comparing recipients based on presence or absence of vascular invasion.

Figure 4. Kaplan-Meier recurrence-free survival curves with 1-, 3-, and 5-year estimates comparing recipients based on the AJCC pathological T stage.

Figure 5. Forrest plot demonstrating multivariate predictors of posttransplant HCC recurrence in descending order of hazard ratios (HR). * HR per log SD increase for NLR and pretransplant maximum AFP. ** HR per SD increase of total cholesterol.

Figure 6. Plot demonstrating posttransplant recurrence risk based on calculated risk score (R). For any given R, the estimated 1-, 3-, and 5-year recurrence risk can be obtained from the corresponding graph. This model had an excellent overall accuracy with a c-statistic of 0.85.

Figure 7. Novel clinicopathologic prognostic nomogram for predicting posttransplant HCC recurrence. For each predictor, a straight upward line is drawn to determine the points accrued. The cumulative points are plotted on the total points bar, and a straight downward line yields the

1-, 3-, and 5-year estimated posttransplant recurrence risk. Nuclear grade: 1= grade 1-2, well differentiated, 2= grade 2-3, moderately to poorly differentiated, 3= grade 4, poorly differentiated, 4= unknown grade due to complete tumor necrosis on pathology. Vascular invasion: 1=none, 2=microvascular, 3=macrovascular. Downstaging: 1=Within Milan or downstaged to Milan, 2= Outside Milan and not downstaged. Radiologic maximum tumor diameter: 1= incidental, 2=non-incidental < 5cm, 3=non-incidental > 5cm. AFP, NLR, and total cholesterol plotted on original scale.